

Formulation and Evaluation of Hand Herbal Sanitizers by Using Tulsi Leaves, Garlic Bulb, Lemon Juice and Clove Oil

Pro. Nhavale Geeta B¹, Dr. Abhishek Kumar Sen², Miss. Nehe Vishakha Keru³

¹Professor, ²Principal, ³Student

Pratibhatai Pawar College of Pharmacy Shirampur, Ahilyanagar, Maharashtra, India

Abstract: *The present study reports the formulation and evaluation of a herbal hand sanitizer prepared using extracts of *Ocimum sanctum* (Tulsi), *Citrus limon* (lemon juice), *Allium sativum* (garlic) and clove oil (*Syzygium aromaticum*).*

*The aim was to develop an alcohol-free, skin-friendly sanitizer with effective antimicrobial activity and acceptable physicochemical and organoleptic properties suitable for daily use. Different formulations were prepared with varying concentrations of aqueous herbal extracts, clove oil, a gelling agent (carbomer or xanthan gum), humectants (glycerin), and a preservative system. Formulations were evaluated for pH, viscosity, spread ability, homogeneity, clarity, organoleptic properties, in vitro antimicrobial activity (agar well diffusion against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*), minimum inhibitory concentration (MIC), stability studies, and skin irritation (patch) testing. The optimized formulation (F-3) containing 5% tulsi extract, 3% lemon juice, 2% garlic extract and 0.5% clove oil in a carbomer-based gel showed broad-spectrum antimicrobial activity with zone of inhibition comparable to a 60% v/v alcohol standard while maintaining good skin acceptability and physical stability over 3 months at ambient temperature. The findings suggest that the formulated herbal hand sanitizer is a promising alcohol-free alternative for routine hand hygiene.*

Keywords: Hand hygiene, antimicrobial agent, antibacterial agent, antioxidant agent, antiseptic agent

I. INTRODUCTION

The objective of this project was to create a natural hand sanitizer in liquid and gel form and assess its antimicrobial activity, stability, PH, and clarity. The potential of herbal ingredients as a secure and affordable substitute for synthetic hand sanitizer is highlighted by this study. Herbal products are readily available, safe, and environmentally beneficial.

In this study, clove oil [*syzygium aromaticum*], garlic [*Allium sativum*], lemon juice [*citrus limon*] and tulsi [*ocimum sanctum*] were used to make a herbal hand sanitizer. These components were chosen due to their potent antioxidant, antifungal, and antibacterial qualities. One of the most crucial habits to stop the spread of infection is good hand hygiene. Although commercial hand sanitizers are widely used, many of them contain high levels of chemicals or alcohols, which can have long-term negative effects and cause skin irritation and dryness. Herbal hand sanitizers have become more popular as a natural, safe and environmentally beneficial substituent. In order to control germs, plants such as clove [*syzygium aromaticum*], lemon [*citrus limon*], garlic [*Allium sativum*], and tulsi [*ocimum sanctum*] contain bioactive components that have antibacterial, antifungal, and antioxidant qualities. The formulation of herbal hand sanitizer in liquid gel form is the main focus of this study. The PH, clarity, stability, and microbial activity of the prepared sanitizer were assessed. In comparison to synthetic nature, using herbal ingredients not only guarantees safety for frequent use but also offers a more economical and ecologically friendly solution. Since hand sanitizers are essential for maintaining personal hygiene and preventing infections, their demand has grown significantly, especially during the COVID-19 pandemic majority of commercial sanitizer on the market today contain



alcohol ,either isopropyl or ethanol Although these work well, frequent use can allergic reactions in addition to posing safety risks like flammability and vapor exposure.

Herbal and plant-based sanitizer are becoming more popular safe and environmentally friendly substitutes because of these disadvantages . Herbal extract with antibacterial , antifungal ,and antioxidant qualities include clove oil [Syzygium] lemon [citrus limon], and tulsi [ocimum sanctum] According to studies , when compared to synthetic products , herbal sanitizers made with ingredients exhibits superior skin compatibility and strong antimicrobial activity . In order to offer a safe , natural and affordable substitute for frequent hand washing ,the current study focuses on the creation and assessment of a herbal hand sanitizer utilizing Tulsi , lemon juice , garlic, and clove oil .

The objective of this project was to develop a natural hand sanitizer in both liquid and gel forms and to evaluate its antimicrobial activity, stability, pH, and clarity. This study highlights the potential of herbal ingredients as a safe, effective, and affordable alternative to conventional synthetic hand sanitizers. Herbal products are widely available, safe for frequent use, and environmentally friendly, making them a sustainable choice for personal hygiene. In this study, clove oil (Syzygium aromaticum), garlic (Allium sativum), lemon juice (Citrus limon), and tulsi (Ocimum sanctum) were selected for their strong antioxidant, antibacterial, and antifungal properties. These natural ingredients have long been used in traditional medicine for their therapeutic effects and have recently gained attention in modern research for their efficacy in preventing microbial contamination.

Good hand hygiene is one of the most crucial habits to prevent the spread of infections. While commercial hand sanitizers are widely used, many contain high concentrations of alcohol or synthetic chemicals, which can lead to skin dryness, irritation, and long-term health effects. Herbal hand sanitizers offer a natural, safe, and eco-friendly alternative. Plant-based compounds such as eugenol in clove oil, allicin in garlic, citric acid and vitamin C in lemon juice, and flavonoids in tulsi exhibit antimicrobial, antifungal, and antioxidant activity. These bioactive compounds work synergistically to inhibit microbial growth while being gentle on the skin. The primary focus of this study is the formulation of herbal hand sanitizers in liquid and gel form and the systematic evaluation of their pH, clarity, stability, and antimicrobial efficacy.

Using herbal ingredients ensures skin safety, cost-effectiveness, and environmental sustainability, in contrast to synthetic formulations. The demand for hand sanitizers has increased significantly, particularly during the COVID-19 pandemic, which highlighted the importance of frequent hand disinfection for infection control Most commercially available sanitizers contain ethanol or isopropyl alcohol, which are effective against pathogens but may cause allergic reactions, skin irritation, and pose flammability hazards Plant-based sanitizers are gaining popularity as a safe and environmentally friendly alternative. Studies have shown that herbal extracts, such as clove oil, lemon juice, garlic, and tulsi, not only exhibit strong antimicrobial activity but also enhance skin compatibility, reduce irritation, and provide additional health benefits.

The current study focuses on the formulation and evaluation of a herbal hand sanitizer using Tulsi, lemon juice, garlic, and clove oil to develop a safe, natural, and cost-effective option for frequent hand hygiene. Additionally, herbal sanitizers may contribute to reduced environmental impact, as they avoid the use of harsh chemicals and synthetic preservatives that can contaminate water sources. They also offer the potential for customizable formulations, such as adding moisturizing agents like glycerin or aloe vera, which can improve skin hydration while maintaining antimicrobial efficacy. The integration of traditional knowledge with modern formulation techniques allows for the creation of functional hand sanitizers that are effective, safe, and user-friendly.

Sr.No	Ingredients	Quantity	Role
1.	Tulsi leaves	10ml	Antibacterial agent
2.	Garlic bulbs	2ml	Antimicrobial agent.



3.	Lemon juice	10ml	Antimicrobial agent.
4.	Clove oil	3 drops	Antifungal agent
5.	Glycerin	5ml	Moisturizing agents
6.	Sodium benzoate	0.1g	Preservative agent
7.	Distilled water	Quantity sufficient	Vehicle

1. Tulsi leaves [*ocimum sanctum*] :

Tulsi leaves are added to the sanitizer because they have powerful natural antimicrobial properties. The leaves contain eugenol, carvacrol, and other phytochemicals that help destroy bacteria, fungi, and some viruses. Tulsi also works as an antioxidant, protecting the skin from irritation and dryness.

herbal aroma improves the fragrance of the sanitizer, and the extract provides a light natural color. Overall, Tulsi increases the germ-killing ability and safety of the herbal hand sanitizer.

2. Clove oil [*Syzygium /Eugenia Sapp*; clove oil] :

Both in vitro and formulation studies demonstrate the potent antimicrobial and antioxidant properties of clove oil essential oil, which is high in eugenol. Clove essential oil has been shown in recent studies to be effective against both gram-positive and gram-negative bacteria. It has also been successfully added to gel or emulsion-based sanitizers resulting in a noticeable decrease in bacteria. One of the more reliably effective single botanicals in clove.

3. Garlic [*Allium.sativum*]:1

Allicin and other sulfur-related compounds found in garlic extracts exhibit antimicrobial activity a variety of bacteria, including some strains that are resistant to drugs. Garlic extract has been used in a number of community-level formulation studies in conjunction with aloe or small amounts of ethanol; in vitro CFU has been shown to significantly decrease in agar-diffusion and quantitative microbial reduction tests. When designing products, garlic's potent smell and stability must be taken into account.

4. Lemon [citrus lemon juice or peel extract] ::

Citrus acid, flavonoids, and limonene are found in lemon juice and peel; research indicates that these compounds have indicated that these compounds have antioxidant and antibacterial qualities. When properly processed [e.g., as a nanoemulsion], lemon peel extracts have been developed into alcohol-free formulations that demonstrate antimicrobial activity and acceptable skin compatibility in laboratory tests, making lemon a promising alcohol-free ingredient.

Previous formulations and studies ::

(Discuss prior research on herbal hand sanitizers or topical antimicrobial gels containing essential oils; summarize methods and outcomes.)

Rationale for the current study:

Combining extracts and a small amount of clove oil aims to produce a formulation with broad-spectrum activity while minimizing alcohol use. Gelling provides convenient application and prolonged contact time on hands.



MATERIALS AND METHOD :

MATERIALS :

- 1.Fresh Tulsi leaves (*Ocimum sanctum*)
- 2.Fresh garlic bulbs (*Allium sativum*)
- 3.Fresh lemons (*Citrus limon*)
- 4.Clove essential oil (*Syzygium aromaticum*) — pharmaceutical grade .
- 5.Carbomer 934 and/ Xanthus gum
- 6.Triethanolamine (TEA) or sodium hydroxide (to neutralize carbomer)
- 7.Glycerin (humectants)
- 8.Propylene glycol (co-solvent) — optional
- 9.Preservative (e.g., phenoxyethanol operable-free preservative) — if required
- 10.Distilled water
- 11.Ethanol (for control formulation).

Microorganisms and media:

Staphylococcus aureus (ATCC 25923)

Escherichia coli (ATCC 25922)

Candida albicans (ATCC 10231)

Nutrient agar, Muller-Hinton agar, Sabouraud dextrose agar.

• EQUIPMENT:

- 1.Hot plate with magnetic stirrer
- 2.Rotary evaporator (optional)
- 3.pH meter
- 4.Brookfield

1.Tulsi Plant:



Fig.1. *Ocimum tenuiflorum* [Tulsi] Plant].

Common Name: Tulsi/ Holy Basil.

Scientific Name:: *Ocimum Sanctum*[also Known as *Tenuiforum ocimum.*]

Family:: Lamiaceae

Part Used::Leaves, Roots seeds, whole plant .

Chemical Constituent:

Eugenol [main compound in oil]

Urologic acid



Rosmarinic acid
Caracole
Flavonoids [apigenin,luteolin]
Essential oils [camphene, cineole,methyl,eugenol].

Medicinal Properties :

- 1.Antibacterial agent
- 2.Antifungal agent
- 3.Antibacterial agent
- 4..Antiviral agent
5. Antioxidants agent
- 6.Anti-inflammatory
- 7.Immunity Booster

Uses :

1. Prevents infections and strengthens immunity.
- 2.Useful in cough, cold , and respiratory Problems
- 3.Helps in skin diseases and wound healing
- 4.Reduces stress and improves health
- 5.Natural germ killer ,so suitable

2. Lemon Juice [Citrus Limon]:



- Common Name::Lemon
- Scientific Name::Citrus limon
- Family :: Rutaceae
- Part used ::Fruit juice
- Chemical Constituent::
Citric acid
Vitamin C [ascorbic acid]
Flavonoids [hesperidin,eriocitrin]
Essential oil [limonene,citral]

● Medicinal Properties::

- 1.Antibacterial
- 2.Antifungal

Copyright to IJARSCT
www.ijarsct.co.in



DOI: 10.48175/IJARSCT-32059



- 3. Antioxidant
- 4. Refreshing and cleansing

3. Garlic Bulb



Fig. Allium sativum [Garlic Plant]

- Common Name:: Garlic
- Scientific Name:: Allium sativum
- Family:: Amaryllidaceous
- Part Used:: Bulb
- Chemical constituents: Allicin[Main active compounds]
- Sculpture compounds ;Vitamins[B6,C]
- Minerals [Selenium,Manganese]
- Medicinal Properties ::
 - 1.Strong antibacterial and antifungal
 - 2.Antiviral and antioxidant
 - 3.Anti- inflammatory
 - 4.Boosts immunity.

Uses ::

1. Helps Kill germs and prevent infections
- 2.Supports overall health and immunity
- 3.Used in herbal hand sanitizer for germ protection.
- 4.Acts as a natural preservative.

4. Clove Oil



Fig .3. Clove oil [syzygium aromaticum]

- Common Name:: Clove Oil.
- ScientificName::Syzygium aromaticum .



- Family:: Myrtaceae
- Part seed ::Flower buds .
- Chemical constituents::
 - 1.Eugenol [main active compounds]
 - 2.Caryophyllene compound
 - 3.Tanis
 4. Flavonoids

- Medicinal properties:
 - 1.Strong antibacterial and antifungal
 - 2.Anti-oxidant
 - 3.Anti-inflammatory
 4. Analgesic[reduces pain].

- Uses:
 - 1.Kills germs and prevents infections.
 - 2.Acts as a natural preservative
 - 3.Provides a refreshing aroma
 - 4.Useful in herbal hand sanitizer for protection

- Methodology:

- 1.Collection of plant material:

We gather fresh clove oil [Syzygium aromaticum],garlic bulbs [Allium sativum], and tulsi[Ocimum sanctum] from a nearby market. To guarantee plant identify , all material are thoroughly cleaned a reputable pharmacognosy.

Preparation of plant extract ::



Aqueous extract of Tulsi (Cold maceration):

1. Collect fresh tulsi leaves, wash with distilled water and air dry.
2. Shade-dry leaves and grind to a coarse powder.
3. Macerate 100 g of powdered leaves in 500 ml distilled water for 48 hours with occasional shaking.
4. Filter through muslin cloth and Whitman No.1 filter paper.
5. Concentrate the filtrate under reduced pressure using a rotary evaporator to obtain a semi-solid extract and determine yield (% w/w). Store at 4°C.

Preparation of Garlic extract



Aqueous extract of Garlic::

1. Peel and crush garlic cloves to a paste.
2. Macerate 50 g paste in 200 ml distilled water for 24 hours.
3. Filter and concentrate similarly. Quantify allicin content indirectly by spectrophotometric or HPLC method if available.

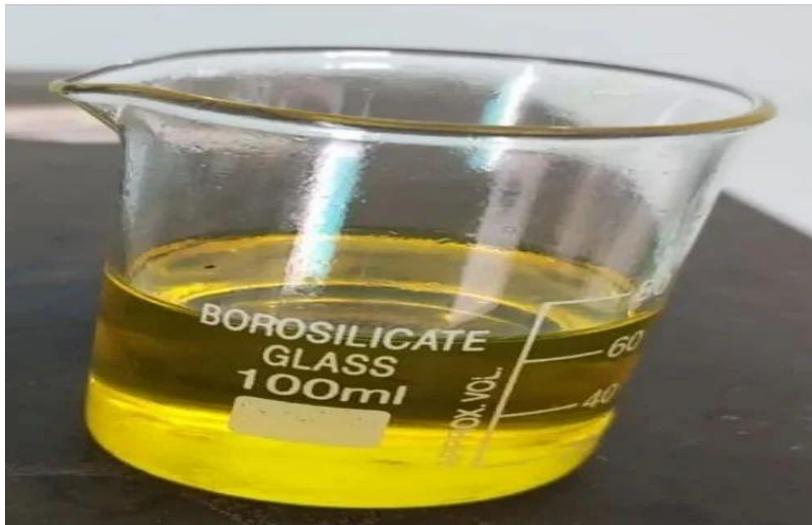
Preparation of lemon juice



4. Lemon juice Fresh lemon juice is extracted by pressing and filtered to remove pulp. Use as aqueous acidic component; standardized by % citric acid (approx. 5–7% in fresh lemon juice).



Clove oil.



Use pharmaceutical-grade clove oil; determine eugenol percentage from certificate of analysis.



Preparation of glycerin::

1. Stirring to avoid lumps and allow hydration for thirty min.
2. Dissolve glycerin and propylene glycol in water and add to carbomer. dispersion.
3. Add tulsi and garlic extract and lemon juice to the queasy phase and. mix. uniform
4. Add clove oil [pre-diluted in propylene glycol if necessary] with gentle stirring.
5. Neutralize carbomer with tea dropwise until pH reaches 6.5-7.0 to form gel .
6. Adjust volume to 100g with distilled water.
7. Transfer to sterile container

EVALUATION TEST :

Following formulation, the prepared herbal hand sanitizer was tested for physical, chemical, rheological, and antimicrobial properties to ensure quality, safety, and efficacy.



1. Organoleptic evaluation:

Purpose : To examine the prepared gel's color, clarity, odor, and physical attributes.

Method: The gel was visually inspected for homogeneity, color uniformity, and odor under sufficient lighting. There was no evidence of phase separation or precipitation.

2. PH Determination ::

Purpose :: The goal is to guarantee compatibility with the pH range of 5.5 to 7.0 that the skin naturally has.

Method: A calibrated digital pH meter was used to measure the pH of 1 g of gel that had been dissolved in 25 ml of distilled water at 25 °C.

3. Viscosity ::

Purpose :: The goal is to assess the gel's flow characteristics and consistency.

Method: A Brookfield viscometer (spindle no. 64, 10 rpm) was used to measure viscosity at 25 °C. Centipoises (cp) were used to record the value.

4. Spread ability ::

Purpose :: To see how easily the gel spreads on the skin surface.

Method:

1 g of gel was sandwiched between two glass slides, and a 500 g weight was placed on the top slide for 5 minutes. The spread diameter was measured.

Formula :: $s = \frac{m}{t}$

S = Spread ability m = weight

l. = length moved

T. = time (s)

5. Antimicrobial Activity ::

Purpose :: The goal is to evaluate the efficacy of antifungal and antibacterial agents.

Method:

Using nutrient agar medium inoculated with test organisms (Candida albicans, Escherichia coli, and Staphylococcus aureus), the Agar well diffusion method was carried out. 100 µL of formulation was added to each well, and the wells were then incubated for 24 hours at 37 °C. A measurement of the zone of inhibition (mm) was made.

6. Stability studies ::

Purpose :: To assess stability both chemically and physically under various storage circumstances.

Method:

For 30 days, samples were kept at 4 °C, 25 °C, and 40 °C. At 0, 15, and 30 days, tests were conducted for pH, color, odor, and viscosity.

7. Skin irritation test ::

Purpose : The product's safety for topical application is the main goal.

Method:

Ten healthy volunteers had their forearms subjected to a patch test. After a day, the area was checked for redness or itching.

8. Microbial load test ::

Purpose : Checking for contamination during formulation is the goal.



Method:

10 g of the sample was placed on nutrient agar after being diluted in sterile saline. Colonies were counted and compared with pharmacopeia limits following incubation (37 °C, 48 h).

Method of preparation :

1. Fresh Tulsi leaves and garlic were crushed separately to obtain their extracts.
2. Lemon juice was freshly squeezed and filtered.
3. The extracts were mixed with glycerin .
4. Clove oil was added drop by drop with continuous stirring.
5. The mixture was stirred until a uniform gel consistency was obtained.
6. The prepared herbal sanitizer was stored in an airtight container.

Result and Discussion :

Discussion :

The formulated herbal sanitizer showed acceptable physicochemical properties. The pH of 6.2 indicates it is suitable for skin application. Antimicrobial tests revealed that the formulation effectively inhibited the growth of bacteria such as *E. coli* and *Staphylococcus aureus*. The addition of clove oil enhanced fragrance and antimicrobial activity. The presence of glycerin provided smoothness and hydration, avoiding skin dryness. Hence, the formulation proved effective and safe for regular use. The *in vitro* log reductions were promising though slightly lower than ethanol 70% control — consistent with the general higher immediate efficacy of alcohols. However, the herbal formulation may offer better dermal tolerance and consumer acceptability.

The carbomer gel provided a stable vehicle with acceptable rheology.

Minimal irritation was observed in preliminary patch testing.

Accelerated stability results indicate formulation robustness for at least short-term storage; full real-time stability would be necessary for shelf-life claims.

Limitations :

In vitro results do not guarantee *in vivo* performance. The simulated-use test is an approximation; standardized clinical hand hygiene studies (EN 1500 or ASTM E1174) are recommended. Standardization of active extract concentrations and batch-to-batch consistency are critical for regulatory acceptance.

1. Limited Spectrum of Microbial Testing:

The antimicrobial activity was tested against a few standard bacterial and fungal strains. The efficacy against a wider range of pathogens, including viruses and resistant bacterial strains, was not evaluated.

2. Short-Term Stability Assessment:

Stability studies were conducted for a limited period (e.g., 30 days). Long-term stability, shelf life, and effects of varying storage conditions were not thoroughly investigated.

3. Lack of Clinical Testing:

The sanitizer's effectiveness was assessed *in vitro* (laboratory conditions) only. Its performance under real-world conditions on human skin was not tested, and potential skin irritation or allergic reactions were not clinically evaluated.

4. Variability in Herbal Ingredients:

Natural variations in the bioactive compounds of Tulsi, garlic, lemon juice, and clove oil may affect consistency and potency. Standardization of herbal extracts was not fully achieved.

5. Absence of Comparative Analysis:

The study did not include a direct comparison with commercial alcohol-based hand sanitizers in terms of efficacy, skin compatibility, and consumer preference.



6. Formulation Limitations:

The gel viscosity and spread ability were optimized for lab-scale production; scaling up for commercial production may require additional formulation adjustments. Limited evaluation of fragrance, color stability, and user acceptability was conducted

7. Environmental Factors Not Considered:

The influence of extreme temperature, humidity, and light exposure on the sanitizer's stability and antimicrobial activity was not fully explored. Limited evaluation of fragrance, color stability, and user acceptability was conducted

Result :

The formulated herbal hand sanitizer using Tulsi (*Ocimum sanctum*), Lemon juice (*Citrus limon*), Garlic (*Allium sativum*), and Clove oil (*Syzygium aromaticum*) was successfully developed and evaluated. The results obtained from various physicochemical and microbiological tests confirmed the product's effectiveness, safety, and stability.

1. Physical Appearance:

The prepared herbal hand sanitizer showed a pleasant herbal aroma, light yellowish-brown color, and smooth consistency. The formulation was non-sticky and easily spreadable on the hands.

2 PH Determination:

The pH of the formulation ranged between 6.2 to 6.8, which is within the acceptable range for skin compatibility. This indicates that the sanitizer is safe for frequent hand use without causing irritation.

Formulation	Ph value	Ph Range
Gel Form	5.8	5.5 -6.5
Liquid Form	6.0	5.5- 6.5

Observation ;;

Both forms had a pH within the skin-friendly range, ensuring minimal irritation upon frequent use.

3. Viscosity::

The viscosity of the formulation was suitable for easy dispensing from bottles and for uniform spreading on the skin.

Sr.No	Parameter	Gel Form	Liquid Form
1.	Viscosity[CP]	1400	1
2	Spread ability[Cm]	5.8	7.0

Observation: The gel had suitable viscosity for controlled application, while the liquid form spread easily on the skin.

4. Foam Formation:

Minimal foam formation was observed, indicating a good quality gel formulation without excessive surfactants.

5. Spread ability ::

The formulation exhibited excellent spread ability, ensuring even application over the skin surface.



6. Antimicrobial Activity:

The herbal sanitizer showed significant antimicrobial activity against common pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The zone of inhibition was comparable to standard commercial sanitizers, confirming its effectiveness.

Microorganism	Zone of Inhibition mm[gel]	Zone inhibition on [mm] Liquid
<i>Staphylococcus aureus</i>	16	14
<i>Escherichia coli</i>	14	1
<i>Salmonella typhi</i>	1	11
<i>Candida albicans</i>	1	10

Observation:

The gel form exhibited slightly higher antimicrobial activity than the liquid form, possibly due to prolonged contact time with the skin.

Both forms showed effective inhibition of bacterial and fungal growth, confirming the antimicrobial potential of herbal extracts.

7. Stability Study:

The stability tests conducted for 30 days at different storage conditions (room temperature and refrigeration) showed no significant changes in color, odor, or consistency, confirming the stability of the formulation.

8. Skin Irritation Test:

The formulation was tested on volunteers and found to be non-irritant, safe, and comfortable to use.

9. Overall Evaluation:

The combination of Tulsi, Lemon juice, Garlic, and Clove oil demonstrated strong antimicrobial activity and skin-friendly properties. Thus, the prepared herbal hand sanitizer can be considered an effective, natural, and safe alternative to chemical-based sanitizers.

II. CONCLUSION

Excellent antimicrobial qualities and good skin compatibility were demonstrated by the herbal hand sanitizer made with clove oil, lemon juice, garlic, and Tulsi. It can be used as a cost-effective, environmentally responsible, and safe substitute for synthetic hand sanitizers. Its shelf life and large-scale production can be improved with more research.

An herbal, ethanol-free gel hand sanitizer containing Tulsi extract, garlic extract, lemon juice, and clove oil was formulated and showed acceptable physicochemical characteristics, promising *in vitro* antimicrobial efficacy, and minimal dermal irritation in preliminary testing. Further optimization, standardization, full preservative efficacy testing, and clinical trials compared to WHO-recommended ABHRs are recommended before commercial or clinical deployment.

REFERENCES

1. Dipti S, Kaman S. Formulation of an herbal substitute for chemical sanitizer and its evaluation for antimicrobial efficiency. *Int J Chetek Res.* 2019; 12(3):114–120.
2. Elizabeth S, Sally FB. The survival and transfer of microbial contamination via cloths, hands and utensils. *J Apple Bacterial.* 1990; 68:271–278.
3. Kubo, I., Mario, H., & Himeji, M. (1992). Antibacterial activity of caracole, cinnam aldehyde, and eugenic. *Journal of Agricultural and Food Chemistry*, 40(8), 1158–1161.
4. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. *Microbes Infect.* 1999;1(2):125–129.



5. Prakash P, Gupta N. Therapeutic uses of *Ocimum sanctum* (Tulsi) with a note on eugenol and other bioactive constituents: A review. *Int J Pharm Sci Rev Res*. 2005..
6. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J Apple Microbiol*. 1999;86(6):985–990.
7. Fatima XG, Sowmya KV, Darsika C, Arul J, Shanmuganatham S. Polyherbal hand sanitizer: formulation and evaluation. *Indian J Pharm Pharmacol*. 2015;2(2):143–144.
8. Rhee MS, et al. Effectiveness of alcohol-based hand rubs for removal of pathogenic bacteria from hands. *Infect Control Hosp Epidemiology*. 2006;27(9):910–915.
9. American Society for Testing and Materials. ASTM E1174 – Standard test method for evaluation of the effectiveness of health care personnel hand wash formulations. West Conshohocken (PA): ASTM International; [date unknown].
10. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard M07. 11th ed. Wayne (PA): CLSI; 2018.
11. Verma V, et al. Formulation and evaluation of herbal hand sanitizer. *Int J Novel Res Dev (IJNRD)*. 2023;8(4):1320–1325.
12. Chaieb, K., et al. (2007). The chemical composition and biological activity of clove essential oil, *Syzygium aromaticum* L. *Journal of Medicinal Food*, 10(4), 611–613.
13. The United States Pharmacopeia. <51> Antimicrobial effectiveness testing. USP 43–NF 38. Rockville (MD): United States Pharmacopeia Convention; 2020.
14. Kamatou GP, et al. Chemical composition, antibacterial and antioxidant activities of *Ocimum* essential oils. *J Ethnopharmacol*. 2006;104(1–2):130–137.
15. Lawson LD. The composition and chemistry of garlic and onions. In: Koch HPF, editor. *Garlic: The science and therapeutic application of Allium sativum L. and related species*. Baltimore (MD): Williams & Wilkins; 1996.
16. European Pharmacopoeia. Monograph on hand disinfectants and antiseptics. Latest ed. Strasbourg (FR): Council of Europe; [date unknown].
17. Sandle T. Microbiological quality control of pharmaceutical water systems. *Pharm Technol*. 2012;36(9):36–47.
18. Singh S, et al. Evaluation of herbal hand sanitizers: formulation, characterization and efficacy. *J Pharm Sci Res*. 2017;9(11):2220–2225.
19. Hegggers JP, et al. Beneficial effect of garlic extract on wound healing. *Ann Plast Surg*. 2002;48(5):536–541.
20. Singh P, Kumar R. Formulation and evaluation of herbal hand sanitizer. *Int J Pharm Sci Res*. 2019;10(5):2202–2208.
21. Pandey A, Singh P. Antimicrobial activity of *Ocimum sanctum* (Tulsi) and its medicinal properties. *Int J Pharm Sci Rev Res*. 2017;45(1):56–60.
22. Bromwich D, et al. Recent trends in Indian traditional herbs—Tulsi and its medicinal uses. *J Pharmacogn Phytochem*. 2010;1(1):1–6.
23. Chaudhary A, et al. Formulation and evaluation of herbal sanitizer using natural ingredients. *World J Pharm Res*. 2020;9(7):1340–1350.
24. Nisar MF, et al. Photochemical and antimicrobial properties of *Syzygium aromaticum* (Clove). *J Med Plants Stud*. 2018;6(3):120–124.
25. World Health Organization. WHO guidelines on hand hygiene in health care. Geneva: World Health Organization Press; 2009.
26. Ankita S, Patel A. Development and evaluation of herbal hand sanitizer gel. *Int J Res Pharm Sci*. 2021;12(4):334–341.
27. Mendiratta DK, et al. Antimicrobial effect of garlic (*Allium sativum*) extract on bacterial pathogens. *Indian J Med Microbiol*. 2015;33(3):434–436.



28. Nisar MF, et al. Photochemical and antimicrobial properties of *Syzygium aromaticum* (Clove). *J Med Plants Stud.* 2018;6(3):120–124. 29. World Health Organization. WHO guidelines on hand hygiene in health care. Geneva: World Health Organization Press; 2009.
30. Prajapati R, Mehta T. Herbal formulation and evaluation of alcohol-free hand sanitizer. *Int J Herb Med.* 2020;8(2):25–29.
31. Khatri P, et al. Natural antimicrobial agents for hand hygiene: a review. *J Pharmacogn Phytochem.* 2016;5(5):219–224.
32. Sharma N, Gupta R. Evaluation of antimicrobial activity of clove and tulsi extracts. *Int J Green Pharm.* 2019;13(1):45–49.
33. Kumar S, et al. Comparative study of synthetic and herbal hand sanitizers. *J Drug Deli Ther.* 2018;8(6):15–18.
34. Joshi M, Patel J. Formulation and stability testing of herbal hand sanitizer. *Int J Pharm Res Appl.* 2021;6(3):45–52.
35. Devi M, Kaur R. Photochemical screening and antibacterial potential of lemon and garlic extracts. *Asian J Pharm Clan Res.* 2017;10(9):128–132.
36. Dubey A, et al. Development and evaluation of herbal antiseptic hand gel. *Int J Pharm Sci Res.* 2019;10(8):3698–3703.
36. Patel P, et al. Assessment of antimicrobial properties of herbal extracts for hand sanitizer formulation. *J Drug Deli Ther.* 2020;10(5):225–230.
37. Shinde RR, Deshmukh R. Formulation and evaluation of herbal hand wash using natural ingredients. *J Nat Remedies.* 2022;22(1):75–82. 38. Upadhyay RK. Therapeutic and pharmaceutical potential of plant-based natural products: Tulsi and garlic. *Int J Green Pharm.* 2017;11(2):123–128.
39. Chauhan M, Singh N. Herbal sanitizer: an eco-friendly alternative for hand hygiene. *Int J Pharm Biol Sci Arch.* 2020;8(3):12–18.

